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Systematic Review

The association between *HIC1* methylation and ovarian cancer: a meta-analysis

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Abstract

Objective: *HIC1* is a tumor suppressor gene (TSG) located in the 17p13.3 region that encodes a transcriptional repressor. Research published over the past few years indicates that *HIC1* methylation is a critical factor in the oncogenesis of ovarian cancer (OC). However, previous studies had only small sample sizes and thus were unable to reach firm conclusions. **Data Sources**: Therefore, we performed a meta-analysis to further investigate the association between *HIC1* methylation and OC. Studies related to *HIC1* methylation and OC were identified from searches of PubMed, EMBASE, Medline and CNKI. **Methods of Study Selection**: Odds ratios (ORs) and 95% confidence intervals (CIs) were used to assess the association between the two factors. Subgroup analysis and Begg's test were used to evaluate heterogeneity and publication bias. From 591 studies, 7 were selected for meta-analysis and these comprised 455 cases and 278 controls. **Tabulation, Integration and Results**: A significant association between *HIC1* methylation and OC was found under the fixed-effects model (OR = 4.306, 95% CI = 2.846 to 6.515). Subgroup analysis of the control type yielded a less tight association (OR = 4.143, $p = 0.147$, $I^2 = 41.1\%$). Finally, we conducted analysis of the Cancer Genome Atlas (TCGA) data and found higher *HIC1* methylation levels in OC compared to adjacent non-tumor tissue. **Conclusion**: In conclusion, this meta-analysis found that *HIC1* methylation was strongly associated with OC.

Keywords: *HIC1*; Methylation; Ovarian cancer; Meta-analysis; Systematic review

1. Introduction

Ovarian cancer (OC) is a lethal gynecologic malignancy comprised of epithelial cancer in 90% of cases [1,2]. OC is the 5th leading cause of cancer death in women, with 21,990 new cases and 15,460 deaths annually in the US [3]. There are no efficient screening programs for OC and in the early stages these patients are asymptomatic. Hence, cases are generally diagnosed with late stage disease where the cancer has disseminated within the peritoneal cavity and making it is impossible to achieve complete surgical removal [4]. So far, only a small number of risk factors have been identified and these include age and a family history of ovarian and/or breast cancer. Parity and the use of oral contraceptives are likely protective factors [5]. Recently, it was reported that methylation of some tumor suppressor genes may play a significant role in OC, including *BRCA1* [6], *HOXA9* [7], *RASSF1A* [8], *SPARC* [9] and *HIC1* [10].

HIC1 is a tumor suppressor gene (TSG) that encodes a transcriptional repressor widely expressed in normal tissues. It is located in 17p13.3, a region frequently hypermethylated or deleted in many human cancer types [11–16]. *HIC1* is methylated in about one third of OC, suggesting it may have a TSG role in this tumor type [10]. Other studies have also shown strong links between *HIC1* promoter methylation and the development of OC. Reversal of *HIC1* promoter methylation in OC may thus provide a rational basis for the clinical treatment of OC.

Various case-control and cohort studies have demonstrated a role for *HIC1* genetic variants in OC. In the present study, we conducted a meta-analysis of all relevant studies using an updated and powerful statistical method in order to study the association between *HIC1* methylation and OC.

2. Methods and materials

2.1 Search strategy

Four reviewers divided into two groups and then searched four databases independently for articles related to OC and *HIC1* methylation. The two groups combined their results after assessment and discussion. Original articles published up to 2020 were identified by the use of search terms including 'ovarian' AND 'cancer OR tumor' AND '*HIC1*' AND 'methylation'. The search process was performed without restrictions on the publication year or language.

2.2 Inclusion and exclusion criteria

The title, abstract and keywords from 591 studies originally identified by the search (130 from PubMed, 188 from EMBASE, 63 from Medline and 210 from CNKI) were further scanned to identify studies on OC and *HIC1* methylation and to exclude irrelevant studies and reviews. To prevent inclusion bias, studies from the primary screen were downloaded for full-text review. Eligible studies were required to meet the following inclusion criteria: (A) OC pa-

tients were diagnosed by pathology; (B) studies evaluated the association between OC and *HIC1* methylation; (C) the number of OC cases in the study was >20. Studies were eliminated if they met one or more of the following exclusion criteria: (A) reviews; (B) cases only included cell lines and animals; (C) cases without controls. After the removal of duplicates from the databases, the remaining studies were selected for data extraction and quality assessment.

2.3 Data extraction and quality assessment

Information extracted from the eligible studies was as follows: first author, year of publication, country and region of study, study population, sample size, frequency of *HIC1* methylation in case and control groups, cancer stage, methylation detection method, and the tissue type used for controls. The Newcastle-Ottawa Scale (NOS) was used to assess the quality of eligible studies. A study that met one of the 9 items listed above scored one point, with the highest possible score being 9. If a study did not include one of the 9 items, that point was considered to be lost. Only studies that reached a score of 5 points or more were included in the meta-analysis.

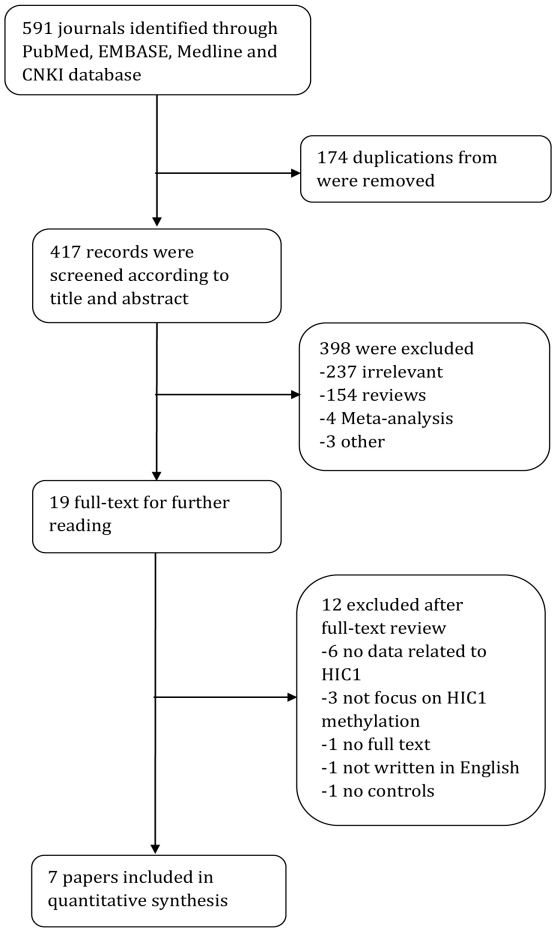

Fig. 1. Flow chart of study selection.

2.4 Statistical analyses

Statistical variables were calculated using STATA (Version.12.0, StataCorp LLC, TX, USA). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to assess the strength of association between *HIC1* methylation and OC. Methylation profiles (Illumina Human Methylation 27) and the corresponding clinical data set for 11 OC cases and 11 controls were downloaded from The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/). Heterogeneity was evaluated with Chi-square test tests and the I^2 value. I^2 = 0–50% indicates no or moderate heterogeneity, while I^2 >50% indicates significant heterogeneity. The fixed-effect model was used if there was no significant heterogeneity, otherwise the random effects model model was used. Subgroup analysis was performed to evaluate the source of heterogeneity. Begg's test and Egger's test were performed to assess the possibility of publication bias. Asymmetry of Begg's funnel plot and a *p*-value in Begg's test of <0.05 were considered to indicate the existence of publication bias.

3. Results

3.1 Study selection

Following the search strategy described above, 591 studies were initially identified from the databases. Two separate review groups independently identified the studies for exclusion and then combined their results. A total of 174 studies were excluded due to being duplicates. After scanning the title and abstract of the remaining 417 studies, 398 were deemed not applicable to this research (237 irrelevant articles, 154 reviews, 4 meta-analyses and 3 others). Next, the full text for the 19 potentially relevant articles was scrutinized. Of these, 6 articles contained no data on *HIC1*, 3 were not focused on *HIC1* methylation, one was not a full text article, one was not written in English and another did not include controls. Following exclusion of these 12 papers, 7 papers were deemed eligible for final quantitative assessment (Fig. 1).

3.2 Description of studies

Relevant information for the 7 studies is presented in Table 1 (Ref. [9,11,16–20]). All were published between 2001 and 2013 and included a total of 733 samples (455 cases and 278 controls). The sample size in each study ranged from 46 to 207 and the majority of patients were aged between 30 and 60 years. Two studies did not record patient age and one study [16] did not report the cancer stage. In the 6 studies that reported cancer stage, there were more early stage patients than advanced stage patients. Cases were either fresh cancer tissues obtained surgically from cancer patients, or archival cancer specimens. All studies used methylation-specific polymerase chain reaction (MSP) for the detection of *HIC1* methylation. The control group was comprised of adjacent tissues (AT) from OC

Table 1. The characteristics of studies.

First author	Publication year	Country	Population	Sample size (Case/Control)	Age (y)	Method	Cancer stage	Control type
[16]	2001	UK	Caucasian	106 (88/18)	NA	MSP	NA	AT
[17]	2002	USA	Caucasian	88 (49/39)	40–79	MSP	I: 3; II: 3; III: 31; IV: 12	BT
[18]	2007	Hong Kong	Yellow	140 (89/51)	NA	MSP	I, II: 32; III, IV: 54	BLT, BT, NT
[19]	2008	USA	Caucasian	207 (100/107)	<50, 50–59, ≥60	MSP	I: 19; II: 2; III: 69; IV: 10	BT, LMP, NT
[9]	2009	China	Yellow	93 (63/30)	(33–66) 53	MSP	I, II: 22; III, IV: 41	AT, NT
[11]	2010	China	Yellow	53 (33/20)	(30–70) 48.7	MSP	I, II: 14; III, IV: 19	BT, NT
[20]	2013	USA	Caucasian	46 (33/13)	(23–79) 57	MSP	I: 15; II: 1; III: 14; IV: 3	NT

NA, not available; MSP, methylation-specific polymerase chain reaction; AT, adjacent tissue; BT, benign tissue; BLT, borderline tissues; NT, normal tissue; LMP, low malignant potential.

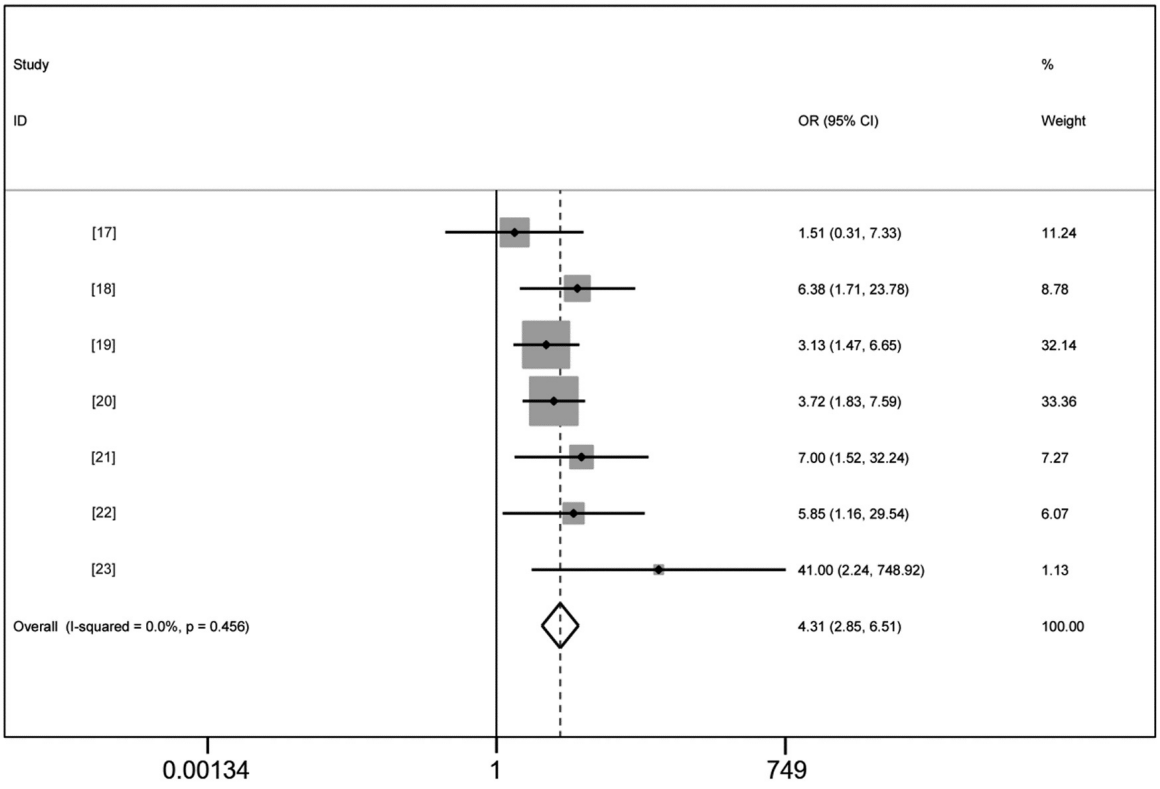

Fig. 2. The frequency of HIC1 methylation was associated with OC.

patients, ovarian tissues from benign OC patients (BT), borderline tissues from OC patients (BLT), normal ovarian tissues (NT) and neoplasia of low malignant potential (LMP).

3.3 Meta-analysis

No significant heterogeneity was found using the Chi-squared and I^2 tests ($p = 0.456$, $I^2 < 0.000$). A fixed-effects model was used to analyze the association between *HIC1* methylation and OC. The forest plot showed the frequency of *HIC1* methylation was strongly associated with OC (OR = 4.306, 95% CI = 2.846 to 6.515) (Fig. 2).

3.4 Subgroup analysis

Subgroup analysis was performed according to the control type. The ORs for the association between *HIC1* methylation and OC were: AT, OR = 2.514; BT, OR = 5.944; BLT, OR = 1.375; NT, OR = 4.931; LMP, OR = 2.061 (Fig. 3). The pooled OR was 4.143 (95% CI: 2.861 to 5.999). The heterogeneity detected for control type was acceptable ($p = 0.147$, $I^2 = 41.1\%$).

3.5 Publication bias

Publication bias was assessed using Begg's test and Egger's test. As shown in Fig. 4, no significant publication bias was found, with the shape of the Begg's funnel plot being approximately symmetrical. The *p*-values from the Egger's test showed no publication bias in any comparison.

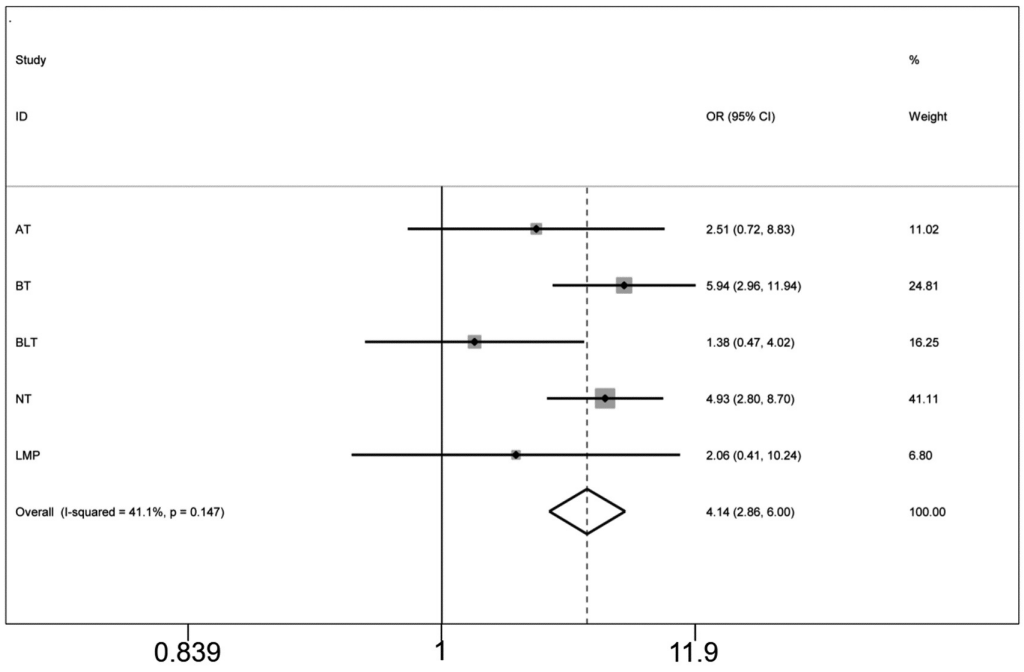

Fig. 3. The ORs for the association between HIC1123 methylation and OC.

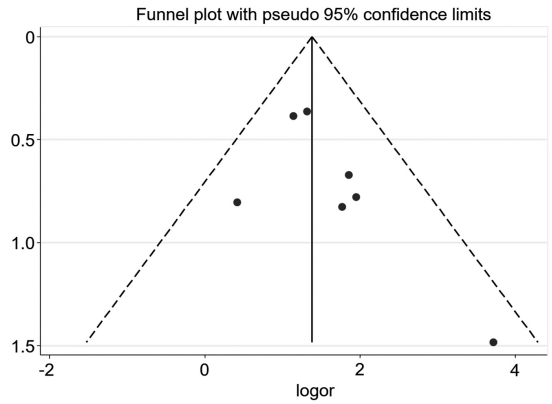

Fig. 4. Analysis for Publication bias.

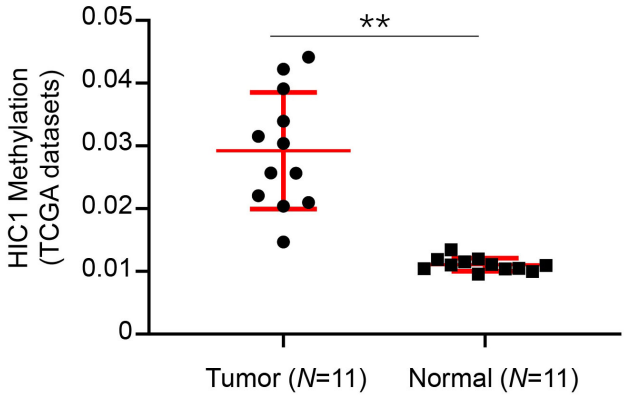

Fig. 5. TCGA data showing *HIC1* methylation in OC tissues and in normal adjacent tissues. Data were shown as mean ± SD. $^{**}p < 0.05$.

3.6 TCGA dataset analysis

To further explore the relationship between *HIC1* methylation and OC, we evaluated publicly available methylation data for OC and adjacent tissues. As shown in Fig. 5, *HIC1* methylation was more frequent in OC tissues than in normal controls ($p < 0.01$).

4. Discussion

OC is one of the leading causes of cancer-related deaths in women worldwide [21]. The identification of markers for early diagnosis and for prognosis is crucial in the clinical treatment of OC. DNA methylation is a common epigenetic alteration that occurs in the promoter region, 5′ and 3′ untranslated regions and exons of genes. Aberrant DNA methylation can inactivate tumor suppressor gene (TSG) function by silencing their expression in various human cancers, thereby promoting tumor development and progression [12,17,22]. Mounting evidence shows that abnormal DNA methylation of TSGs leads to downregulation of gene expression in OC [23,24]. Methylation of the *HIC1* TSG disrupts its normal function and promotes the progression of various cancer types [13,14,25–27].

Since the original report of *HIC1* methylation in OC, discordant conclusions have been reached regarding its association with OC. Tam showed the frequency of *HIC1* methylation in OC tissue was significantly higher than in non-malignant ovarian tissue [18]. Feng suggested that *HIC1* methylation was more frequent in early stage OC compared to late stage OC [19]. However, a study by

Ozdemir *et al*. [28] did not detect *HIC1* methylation in any OC samples, in contrast to the above reports.

In the present work we evaluated 7 studies containing a total of 455 cases and 278 controls in order to investigate the relationship between *HIC1* methylation and OC. In this meta-analysis, *HIC1* methylation was strongly associated with OC (OR = 4.306, 95% CI = 2.846 to 6.515). Together with the results from subgroup analyses, we showed the *HIC1* methylation level in OC cases was significantly higher than in controls. These results did not appear to be affected by publication bias. Moreover, results from the TCGA database also showed the *HIC1* methylation level in OC tissue was significantly higher than in adjacent, non-tumor tissues. The present work indicates that *HIC1* methylation is strongly linked with OC, and is consistent with the results of previous studies [18–20].

Although we have identified an association between *HIC1* methylation and OC, this study has several limitations. Firstly, the molecular mechanism by which *HIC1* methylation is linked to the development of OC is still not fully understood. Secondly, the small number of studies limited the quality of meta-analysis and could affect the strength of the conclusion. The observed association could also be influenced by multiple confounding factors such as hormonal therapy, nulliparity, environmental factors and family history of OC. Finally, although no significant publication bias was found using the Begg's test and Egger's test, it is possible that negative and unpublished investigations may contribute some bias.

In conclusion, our analysis showed that *HIC1* methylation was significantly associated with OC, thus providing a potential biomarker for the early diagnosis of OC, as well as a potential prognostic indicator. Further studies with sufficiently large sample size are needed to confirm the link between *HIC1* methylation and OC before clinical application.

Author contributions

JG concepted and wrote an original draft. LFS concepted, supervised review and edited the draft. QQL participated in the experiment design and analysis. All authors contributed to editorial changes in the manuscript. All the authors read and approved the final manuscript.

Ethics approval and consent to participate

All subjects gave their informed consent for inclusion before they participated in the study. This study was approved by the Ethics Committee of Beijing Jinshuitan Hospital with approval number KW-288731.

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Conflict of interest

The authors declare no conflict of interest.

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